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Reproductive Behavior of Cloned Gametophytes of *Pteridium aquilinum* (L.) Kuhn.

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ABSTRACT.—Spores from single fronds of three different taxa of *Pteridium aquilinum* (L.) Kuhn were collected at different sites in Scotland, England and Sri Lanka. Gametophytes developed from these spores were treated to produce arrays of genetically identical clones. Sporophyte formation was determined when such clones from the same or different gametophytes, derived from the same frond, were combined in pairs in all possible ways to produce a diallel mating scheme. A recurring pattern of presence or absence of sporophyte formation indicated the occurrence of two genetic classes defined by no or very few sporophytes in pairs within either class but high frequency production in pair combinations of clones from different classes. The usual failure of sister clone pairs to produce a sporophyte contrasts with the frequently high incidence of sporophyte formation on the part of single, isolated non-cloned gametophytes. This conflict of evidence is discussed in relation to genetic incompatibility or, alternatively, the control of antheridia formation. The genetic differences revealed in cloned gametophytes provide an empirical way of determining whether a given stand of bracken is made up of more than one individual.

The breeding behavior of bracken gametophytes presents some unresolved problems. Thus, Wilkie (1956) produced experimental evidence for genetic incompatibility in bracken by recording the frequency of sporophyte formation in combinations of clones from different gametophytes derived from single fronds. The clones were produced by harvesting the prothalli which proliferated from the margins of sectioned gametophytes. The results, in clones prepared from three Scottish populations of bracken, could be plausibly reconciled with the occurrence of two mating types in each population. Since combinations of clones between populations were cross-compatible, it appeared that a single locus, multi-allele system was present so that each sporophyte would be heterozygous for dissimilar alleles. It was also noted that, although well defined, the apparent incompatibility was not absolute since a low, variable frequency of sporophytes occurred among putatively incompatible combinations.

On the other hand, Klekowski (1972) reported that single, isolated gametophytes of bracken from different localities display wide variation in sporophyte formation, ranging from zero to nearly 100%, with most samples from different sites exceeding 30%. Self-fertilization in such gametophytes is the rule and incompatibility is conspicuously absent. The differences in frequency of sporophyte formation per sample were attributed to differences in the frequency of recessive sporophytic lethals for which the parent plants were heterozygous. He also noted that Wilkie's findings could be explained if the populations concerned carried balanced lethals, whereby each parent plant would be a double heterozygote for recessive sporophytic lethals at two loci linked in repulsion. Haploid gametophytes would carry one or the other of the

lethals and thereby present the appearance of two mating-types. If the lethality of either homozygous combination were incomplete i.e., the lethals were leaky, apparent cases of breakdown in the incompatibility system, inferred by Wilkie (1956), could be accounted for. Klekowski also indicated the need for further study of Scottish populations that might prove atypical, a suggestion that prompted the present study.

The experiments described here were designed to discover whether the appearance of two "mating-types", whatever their origin, could be detected in bracken populations from Scotland, England and Sri Lanka. It is particularly important to discover whether or not evidence from the British and Sri Lankan populations, geographically separated and belonging to different subspecies, leads to the same conclusions.

Material and Methods

Sample Sites.—Seventeen spore samples were obtained from single fronds collected at the sites indicated in Table 1. Ten Scottish spore collections were obtained from Clunie Dam (CD1 to CD5), Black Hill (BH3 and BH11), Temple (T1), Rubery Reservoir (R1) and Edgelaw Reservoir (E1). Spores were also obtained from one English site and two Sri Lankan sites: Farr's Inn and Bambarakanda Falls. Five samples (SL1 to SL5) were collected at the former and one (SL6) at the latter site.

Three taxa are included in these collections. Both *Pteridium aquilinum* (L.) Kuhn ssp. *aquilinum* and ssp. *fulvum* (Kuhn) Page & Mill. are included in the Clunie Dam samples. CD2, CD3, and CD4 belong to ssp. *fulvum* and CD1, and CD5 to ssp. *aquilinum*. The stand of ssp. *fulvum* is roughly triangular with sides of approximately 20 m (Page and Mill, 1994). Three fronds CD2, CD3, and CD4 were collected approximately 10 m apart from the west side of the stand. The ssp. *aquilinum* fronds, CD1 and CD5 were collected adjacent to, respectively, the north and south sides of the stand of ssp. *fulvum*, which is surrounded by sporophytes belonging to ssp. *aquilinum*. The Black Hill site refers to a roughly circular, isolated stand of ssp. *aquilinum* surrounded by *Calluna* moor. Two fronds were collected 60 m apart. The English population was from a scattered distribution of *aquilinum*.

From the Sri Lankan populations of ssp. *revolutum* (Kuhn) Wu Zheng-yi & Raven, which is common in upland areas and the only subspecies in the island, five fronds were collected over a 15 m distance within a fairly continuous stand bordering one side of a road. The other site, (SL6) is several miles away from Farr's Inn and at a lower elevation by some 1500 m.

Culture of Gametophytes.—Spores were collected overnight by inverting fertile fronds on paper. The spores were washed three times by centrifugation in sterile water. Single drops of suspended spores were transferred by micro-pipette to petri dishes with 1% agar (Sigma A7002) made up with Knop's solution and sterile water (Wilkie, 1956). All cultures were kept at 20°C under a standard fluorescent strip light, except for occasional periods under daylight

TABLE 1. Locations of the different single-frond spore collections from three sub-species of *Pteridium aquilinum* (L.) Kuhn. Nomenclature for British samples follows Page (1982) and Page and Mill, (1994), and for the Sri Lankan samples Wu Zheng-yi and Raven (1999). Map references for British samples refer to the U.K. Ordnance Survey, Landranger Series.

Site	Identification of spore collections	Map reference
ssp. *aquilinum*		
Clunie Dam	CD1, CD5	GR NN 915 592
Black Hill	BH3, BH1	GR NT 184 636
Rubery Reservoir	R1	GR NT 311 571
Edgelaw Reservoir	E1	GR NT 306 581
Temple	T1	GR NT 312 583
Hutton-le-Hole	Y1	GR SE 700 890
ssp. *fulvum*		
Clunie Dam	CD2, CD3, CD4	GR NN 915 592
ssp. *revolutum*		
Farr's Inn	SL1, SL2, SL3, SL4, SL5	80 E 49 6 E 49
Bambarakanda Falls	SL6	80 E 51 6 N 46

and ambient temperature, which applied equally to all cultures within a set of comparisons.

The frequency of sporophyte formation by gametophytes was recorded under the following conditions. When thalli had grown to about 0.5 cm diameter, a random sample was transferred individually to small compartments, 2 cm square and 1.7 cm deep, in plastic boxes made up of 25 such compartments, each provided with sterile, washed sand moistened with Knop's solution. The appearance of a sporophyte was attributed to self-fertilization. Pairs of such gametophytes, whose members were from different sites, were also kept under similar conditions. Any sporophytes which appeared in the latter comparisons may have arisen by selfing or crossing between gametophytes.

A different kind of experiment was carried out with cloned gametophytes derived from single thalli. This entailed the combining of the cloned gametophytes in pairs, either according to a regular scheme described below, or randomly combined within or between different spore samples.

To produce clones, young spore derived gametophytes were either treated for five minutes with 0.5 M KCl (Dyer, 1979) and then washed with water or they were cut into segments. Most, but not all, gametophytes treated either way and kept thereafter on Knop's agar substrate produced many small thalli around the margins. These small thalli were removed and grown to produce arrays of genetically identical clones. For each sample of spores from a given collection, 25 randomly chosen gametophytes were used to produce clones. Either method of producing clones led to the same conclusions. Treated gametophytes differed in the rate of formation of daughter clones. When twenty or more clones became available, for at least nine or ten treated gametophytes, the clones were removed to set up the experiments described

TABLE 2. Diallel combinations of cloned gametophytes. The numbers 2 and 4 refer to the potential maximum number of sporophytes for combinations of clones from, respectively, the same or a different gametophyte. Combinations between identical clones occur once, but twice between clones from different gametophytes.

Clone numbers										
1	2	3	4	5	6	7	8	9	10	
2	4	4	4	4	4	4	4	4	4	1
	2	4	4	4	4	4	4	4	4	2
		2	4	4	4	4	4	4	4	3
			2	4	4	4	4	4	4	4
				2	4	4	4	4	4	5
					2	4	4	4	4	6
						2	4	4	4	7
							2	4	4	8
								2	4	9
									2	10

below. In the case of CD4, for reasons explained later, a particular test was repeated with sets of clones that had developed later.

To compare the behavior of cloned gametophytes they were transferred in appropriate pairs, when about 0.5 cm in diameter, to individual compartments of the plastic boxes under the conditions noted above. After ten to fourteen days they were irrigated with aerated tap water. This was repeated at intervals until two to three weeks had elapsed without the further appearance of sporophytes, when the experiment was terminated. The presence of sporophytes was determined by inspection with a low-power binocular microscope. To avoid possible damage due to handling and to avoid the risk of contamination, the occurrence of archegonia and antheridia was not followed during these tests. As will be apparent later, that in no way detracts from the significance of the evidence but points to an obvious subject of future enquiry.

ANALYTICAL PROCEDURE.—Genetic incompatibility has been claimed to account for the reproductive behavior of cloned gametophytes. An obvious way to check this is to set up a N × N diallel mating system whereby members of the arrays of clones derived from the same frond are combined in pairs in all possible ways, including combinations between sister clones. This results in the mating scheme illustrated in Table 2. For convenience it can be collapsed into the indicated triangular form. The lower diagonal position (diagonal slots) refers to the pairing of sister clones while all the other positions refer to combinations between the arrays (e.g., 1 × 2, 2 × 1). Since a gametophyte, cloned or otherwise, has the capacity to produce a single sporophyte, the maximum number of sporophytes expected in the diagonal slots is two whereas four are expected for all the other, duplicate combinations shown in the diagram. Interpretation of the reproductive behavior of clones depends on the nature of the departure from the numerical distribution shown in Table 2 when they are combined in such a diallel scheme.

It is first necessary to consider how the presence of a simple genetic incompatibility system would affect the distribution of sporophytes in a diallel test. In the case of two, equally frequent haploid mating types (+ and −) where only the heterozygote (+/−) will give rise to a sporophyte, the results of combining gametophytes from a heterozygous individual can be represented as:

	+	−
+	0	2
−	2	0

or, more succinctly as

	+	−
+	0	4
−		0

Extending the same sort of diagrammatic representation to the simplest hypothesis of diallel combinations in which the gametophyte clones in individual arrays are all (+) or all (−), the results can be ordered to display the characteristic pattern shown in Table 3a. Note there is a rectangular set of positions, representing the heterozygotes, with a maximum number of four sporophytes. All the other positions will fall into one of the two triangles that represent either the (++) or the (−−) homozygotes; these do not produce sporophytes. In a diallel test of this kind the practical task is to see whether the order of the paired gametophytes can be arranged to display the characteristic pattern of sporophyte production.

Exactly the same kind of pattern can be generated if the parent individual is heterozygous for two recessive sporophytic lethal genes at two loci linked in repulsion, in which case only the double heterozygote will give rise to a sporophyte. This balanced lethal situation (Table 3b) leads to the same pattern of sporophyte production as the case of simple incompatibility, provided the linkage is complete. For either hypothesis the model assumes equal numbers of the alternative genotypes among the gametophytes which give rise to the clones used in any diallel test. In practice there will be chance variation about the 1:1 ratio and this will lead to corresponding departure from the precisely symmetrical pattern of the theoretical distribution illustrated in Tables 3a and 3b. Where it is necessary to test for departure from a 1:1 ratio the Chi-Square test has been used.

Results

Sporophyte Production in Non-cloned Gametophytes.—Young gametophytes were removed from the agar plate at random and allowed to develop either on their own (Table 4) or in the proximity of another gametophyte derived from a different frond of the same or a different taxon (Table 5). In the first situation the comparisons include samples from ssp. *aquilinum* (CD1, CD5, E1, R1, and T1), from ssp. *fulvum* (CD2, and CD4) and from ssp. *revolutum* (SL1, SL3, SL4, SL5, and SL6). Among these isolated gametophytes, the frequency of sporophyte production ranged from 0.16 to 0.76, with an average of 0.43. These data are consistent with the variation reported by Klekowski (1972). In the second design, the combination of gametophytes from different sources resulted in a much higher frequency of sporophyte production. Almost all (0.95) of a total of 598 such combinations produced at least one and often two

TABLE 3. The potential maximum numbers of sporophytes produced by crossing, in all possible ways, clones from eight different gametophytes derived from a sporophyte heterozygous for either: a) (+) and (−) genotypes or b) balanced lethals linked in repulsion. It is assumed that alternative haploid genotypes are equally frequent in each situation. The diagonals refer to the single combinations of identical clones; all other combinations occur twice.

a) gametophytes derived from a sporophyte heterozygous for (+) and (−) genotypes

Clone numbers and genotype									
1	2	3	4	5	6	7	8		
+	+	+	+	−	−	−	−		
0	0	0	0	4	4	4	4	+	1
	0	0	0	4	4	4	4	+	2
		0	0	4	4	4	4	+	3
			0	4	4	4	4	+	4
				0	0	0	0	−	5
					0	0	0	−	6
						0	0	−	7
							0	−	8

b) gametophytes derived from a sporophyte heterozygous for balanced lethals linked in repulsion

Clone numbers and genotype									
1	2	3	4	5	6	7	8		
+1	+1	+1	+1	1+	1+	1+	1+		
0	0	0	0	4	4	4	4	+1	1
	0	0	0	4	4	4	4	+1	2
		0	0	4	4	4	4	+1	3
			0	4	4	4	4	+1	4
				0	0	0	0	1+	5
					0	0	0	1+	6
						0	0	1+	7
							0	1+	8

sporophytes, whether the combinations were within or between taxa (Table 5). The lower production of sporophytes in isolates suggests a high incidence of recessive, sporophytic lethals at different loci. Given full penetrance of sporophytic lethals and heterozygosity in the source sporophytes for one, two, or three different, independently assorting lethals, frequencies of respectively 0.5, 0.25 and 0.125 are expected in random samples of isolated, selfed gametophytes. All the tests referred to in Table 4 can be reconciled with heterozygosity for either one or two lethals except T1 and SL5 in which the proportion of combinations with a sporophyte significantly exceeds 0.5 ($p < 0.01$). However, for environmental or genetic reasons, not all lethals may be fully expressed when homozygous. Lethals may be "leaky" so that sporopyhte occurrence is higher than would otherwise be predicted, possibly so in T1 and SL5. The high frequency of sporophyte production with gametophytes from different sources (Table 5) is also consistent with the occurrence of recessive lethals at different loci.

TABLE 4. Sporophyte frequency in single, isolated, non-cloned gametophytes derived directly from spores. N refers to the number and + and 0 to those with or without a sporophyte.

Origin	N	+	0	% fertile
ssp. *aquilinum*				
CD1	50	14	36	0.28
CD5	25	15	10	0.60
E1	99	50	49	0.51
R1	100	55	45	0.55
T1	99	66	33	0.67
ssp. *fulvum*				
CD2	50	12	38	0.24
CD4	25	16	9	0.64
ssp. *revolutum*				
SL1	25	7	18	0.28
SL3	25	5	20	0.20
SL4	25	4	21	0.16
SL5	25	19	6	0.76
SL6	25	7	18	0.28

CLONED GAMETOPHYTES OF SSP. *FULVUM*.—Tables 6 and 7 show the diallel combinations for the Clunie Dam samples derived from the stand of *fulvum* (CD2, CD3, and CD4). The original gametophytes used to produce clones were arbitrarily assigned numbers e.g., CD1 to CD25 to identify the clones derived from a particular gametophyte. These are the axes in Tables 6 to 9 and 11 to 13. For each analysis, the combinations have been arranged to best compare the observed distribution of sporophytes with the patterns illustrated in 3a and 3b of Table 3.

The distribution of sporophyte production in Table 6 for pair combinations can be explained by the presence of two genotypes for which the parent frond was heterozygous. When members of a gametophyte pair belong to the same genotype, sporophyte formation does not occur but does so when they differ in this respect. All sister clone pairs, derived from the same gametophyte (the diagonal slots), failed to produce sporophytes and the pattern of presence or absence of sporophytes corresponds to the pattern in both Tables 3a and 3b. Thus, for CD2 clone numbers 1, 4, 5, 6, 15, 2, and 9 did not produce a sporophyte or only rarely did when paired with a member of that set. Similar results are seen for the members of the other set, clone numbers 3, 10 and 14. However, when members of different sets were combined at least one and often three or four sporophytes were produced. Occasionally, one or two sporophytes occur where, according to either the incompatibility or balanced lethal model, none are predicted. The same pattern is encountered with CD3. The two categories or classes of clone include numbers 7, 9, 20 and 21 on the one hand, and numbers 4, 1, 9,12, 16 and 17 on the other, with the same qualifications as noted for CD2.

The diallel test with CD4 was carried out twice (Table 7). In the first test, performed at the same time as the tests with CD2 and CD3, all nine series of

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TABLE 5. Frequency of at least one sporophyte per pair (+) when non-cloned gametophytes from different locations are combined in pairs. In all the Clunie Dam (CD) pairs one gametophyte belongs to *aquilinum* and the other to *fulvum*. The Sri Lankan pairs were derived from putatively different individuals of *revolutum*, while in the remaining combinations (SL3 and BH11) one gametophyte belongs to *aquilinum* and the other to *revolutum*. N refers to the number of pairs.

Pair combinations	N	+	0	% fertile
ssps. *aquilinum* and *fulvum*				
CD1 and CD2	174	170	4	0.97
CD1 and CD3	100	85	15	0.85
CD5 and CD2	25	25	0	1.00
CD5 and CD4	124	122	2	0.98
ssps. *revolutum* and *revolutum*				
SL1 and SL3	25	25	0	1.00
SL1 and SL4	25	25	0	1.00
SL3 and SL5	25	22	3	0.88
SL3 and SL6	25	24	1	0.96
SL4 and SL6	25	22	3	0.88
ssps. *aquilinum* and *revolutum*				
BH11 and SL3	50	48	2	0.95
Total	598	568	30	0.95

clones failed to produce sporophytes or did so only very rarely. As noted above, although the model assumes two equally frequent classes among the gametophytes, derived from the single frond that gave rise to the clones, chance will cause variation about a 1:1 ratio in a random sample. However, it seems improbable, but not impossible, that nine gametophytes would belong to a single genotype.

The test with CD4 was repeated with a second series of clones which had developed later. This second test is in accord with the data from the previous CD2 and CD3 tests, suggesting that the first test with CD4 was non-representative. In the second test, one genotype included clone numbers 10, 14, 23, and 25 while the other included clone numbers 24, 1, 8, 11, 13, and 15. To identify which of the two genotypes is the one represented in the first test, clone numbers 18 and 19 of the first test were combined with all but one of the different clones used in the second test. Table 8 indicates that all the clones used in the first test belong to the same genotype as clone numbers 10, 14, 23, and 25 of the second test. Although unlikely, these data support a departure from a 1:1 ratio of genotypes among the cloned gametophytes of the first test.

It was noted earlier that CD2, CD3, and CD4 were derived from a single stand of ssp. *fulvum*. It is therefore of interest to ascertain the genetic comparability among them. Clones belonging to the alternative genotypes of respectively CD2 and CD3, CD2 and CD4, and CD3 and CD4 were combined in pairs. To compensate for the occasional shortage of replicates, clones not represented in the original test were included e.g., CD3 number 18 and CD4 number 3. One combination was lost due to algal infection. Table 9 indicates identity of the

TABLE 6. Diallel tests with the Clunie Dam samples (CD2 and CD3) of cloned gametophytes of ssp. *fulvum*. The clone numbers have been arranged to reveal the pattern of combinations that do or do not produce sporophytes, as in Tables 3a and 3b.

CD2 clone numbers										
1	4	5	6	15	2	9	3	10	14	
0	0	0	0	0	1	2	1	3	3	1
	0	0	0	0	1	1	3	4	4	4
		0	0	0	0	0	4	3	2	5
			0	0	0	0	3	3	2	6
				0	1	0	4	2	3	15
					0	1	4	3	4	2
						0	4	3	4	9
							0	0	0	3
								0	3	10
									0	14
CD3 clone numbers										
7	19	20	21	4	1	9	12	16	17	
0	0	1	2	2	2	3	3	3	3	7
	0	0	0	3	3	2	3	2	2	19
		0	0	1	2	2	2	4	4	20
			0	3	3	3	3	4	2	21
				0	0	0	0	0	0	4
					0	0	0	0	0	1
						0	1	0	0	9
							0	0	0	12
								0	0	16
									0	17

two genotypes in the three sets of cloned gametophytes. Pairings within genotype failed to produce sporophytes or did so only rarely so whereas pairing between genotypes often yielded the maximum of two sporophytes. By cross referencing, the total number of clones listed in the tests described in Tables 6 to 9 can be assigned to two genotypes comprising 24 and 16 samples respectively. This is not significantly different from a 1:1 ratio ($\chi^2 = 1.6$, $p > 0.1$). This evidence makes it likely that the stand of ssp. *fulvum*, from which CD2, CD3, and CD4 were collected, constitutes a single individual. This conclusion is also consistent with the results of randomly combined, paired, cloned gametophytes either from the same or different fronds from the stand of ssp. *fulvum* (Table 10, Sections i and ii). Among these pairs within fronds there is a 1:1 ratio of combinations that produce sporophytes and those that fail to do so. The same is generally true for combinations of clones derived from different fronds, except for a statistically significant excess of pairs which produce sporophytes when clones belonging to CD2 and CD3 were combined ($\chi^2 = 13.5$, $p < 0.01$). This is in sharp contrast to the combinations of cloned gametophytes between taxa, Sections iii and iv of Table 10. In these combinations, there is a consistently high incidence of sporophyte formation and often the maximum is produced.

TABLE 7. The repeat diallel test with the Clunie Dam sample (CD4).

CD4 i)

2	6	16	17	18	19	20	21	22	
0	0	1	0	0	0	0	0	1	2
	0	0	0	0	0	0	1	0	6
		0	0	0	0	0	0	0	16
			1	0	0	0	0	0	17
				0	0	1	0	0	18
					0	0	0	0	19
						0	0	0	20
							0	0	21
								0	22

CD4 ii)

10	14	23	25	24	1	8	11	13	15	
0	0	0	0	4	3	3	3	2	3	10
	0	0	0	3	4	3	3	3	4	14
		0	1	2	4	4	3	3	1	23
			0	4	3	4	2	4	1	25
				0	0	2	0	0	0	24
					0	0	0	0	0	1
						0	0	0	0	8
							0	0	0	11
								0	0	13
									0	15

CLONED GAMETOPHYTES OF SSP. *AQUILINUM*.—These experiments involve two samples collected adjacent to and on opposite sides of the stand of ssp. *fulvum*. Pairing among clones of either CD1 or CD5 were carried out in the same manner as described for *fulvum* and the results are shown in Table 11. In the CD1 diallel combinations the situation is similar to that already seen in *fulvum*. The diagonal slots are all zero. It appears that clone numbers 1, 3, 7, 11, 14 and possibly 12 belong to one genotype and 2, 5, 8, and 9 belong to the other. In CD5, although there is again evidence for the presence of two genotypes, there are more exceptions to the predicted occurrence of sporophytes. Thus, three of the ten pairings of sister clones give rise to one or two sporophytes. Clone numbers 2, 5, 13, 17, and 10 probably belong to one genotype and clone numbers 11, 12, 20, 4 and 6 to the other.

The two Black Hill samples (Table 12) also differ to some extent from each other. In BH3, clone numbers 1, 3 and 9 appear to belong to one category and numbers 4, 6, 12, 14, and 19 to the other; clone numbers 15 and 20 are exceptions. In their case sister clone pairing leads to the appearance of either one or two sporophytes in the diagonal slots. Also both numbers 15 and 20 produce sporophytes when paired with a member of either of the two sets: (1, 3, and 9) or (4, 6, 12, 14, and 19). In the balanced lethal model, this could occur if linkage is incomplete so that recombination in the parent sporophyte produces gametophytes which do not carry either of the sporophytic lethals. Alternatively, interactions with genes at other loci might be responsible for

TABLE 8. Test of genetic identity among the CD4 clones of *fulvum*. The effect of combining in pairs clone numbers 18 or 19 of the first test with the CD4 clones used in the second diallel test. Each combination was represented by a single pair so the maximum predicted number of sporophytes is two.

CD4 clones of second test									
10	14	23	25	1	8	11	13	15	CD4 clones of first test
0	0	0	0	2	2	1	2	2	18
0	0	0	0	2	1	1	1	2	19

allowing sporophytes to develop when they would not otherwise do so. BH11 behaves like *fulvum* with two categories of cloned gametophytes that distinguish, respectively, clone numbers 11, 21, 25, 22 and 18 from clone numbers 24, 14 and 9. In the small York sample with five sets of clones there is again evidence of two categories, numbers 4, 5 and 16 and numbers 1 and 22.

CLONED GAMETOPHYTES OF SSP. *REVOLUTUM*.—The diallel results for SL1, SL2, SL3, and SL4 are shown in Table 13. All the diagonal slots are empty, except for one exception in both SL2 and SL3, and there is the now familiar pattern of two alternative sets of clones. For SL1, one set or genotype includes clone numbers 3, 4, 5, 6, 8, and 10 and the other includes clone numbers 1 and 2. In SL3 the alternative sets comprise clone numbers 2, 3, 4, 6, 7, 8, 9, and 15, on the one hand, and clone numbers 1 and 5 on the other. In SL4 the alternative sets or genotypes are clone numbers 1, 3, and 4 and clone numbers 5, 2, 6, 7, 8, and 9. None of these distributions depart significantly departs from a 1:1 ratio.

SL2 is inconsistent and illustrates the kind of exception referred to in CD5 and BH3. Thus, six of the clones fall into two categories, numbers 3, 4, and 5 and numbers 1, 7, and 9. Clone numbers 2 and 8 are exceptions. These clones produce sporophytes when combined with a sister clone or a clone belonging to either set (Table 13).

DISCUSSION

Three features of these experiments are of particular significance. Firstly, with very few exceptions, sister clone pairs fail to produce sporophytes. Pooling the data over all tests, only 12 sporophytes formed out of a total of 118 pairs of sister clones. Such infertility is not due to loss of potential to develop the hermaphroditic condition on the part of cloned gametophytes since the pairing of such clones derived from different spores collected at different sites or from different taxa regularly led to a high and often maximum rate of sporophyte production (Table 10).

Secondly, the evidence from the diallel experiments indicates that clones derived from a single frond belong to one of two classes such that, sprorophyte formation depends generally on the joint presence of a cloned gametophyte from each class (Tables 6 to 9 and 11 to 13). It is assumed that the difference between classes is genetic.

TABLE 9. Tests of genetic identity among clones of the Clunie Dam samples of *fulvum* (CD2, CD3, and CD4). Cloned gametophytes are combined in pairs. One combination (1 × 16) was lost due to fungal infection.

CD2 clones										
14	10	3	15	9	6	5	4	1	2	
0	0	1	1	2	1	1	2	1	1	1
1	1	0	1	2	1	2	1	1	1	9
0	1	0	1	2	1	1	1	1	1	12
0	0	0	1	2	2	2	1	-	1	16
0	0	0	2	2	1	1	1	2	1	18
0	1	1	1	2	2	2	2	1	2	4
1	1	1	0	0	1	1	0	0	0	7
1	2	1	0	0	0	0	0	0	1	19
1	1	1	0	0	0	0	0	0	0	20
2	2	2	0	0	0	0	0	0	0	21

CD3 clones

CD2 clones

14	10	3	15	9	6	5	4	1	2	
0	0	0	1	1	2	1	2	2	1	3
0	1	0	2	2	1	2	2	1	2	6
0	0	0	1	2	1	1	2	2	2	16
0	0	0	1	2	2	1	1	2	1	17
0	0	1	2	1	1	1	2	2	1	18
0	0	0	2	1	1	1	2	2	2	19
0	1	0	1	2	1	1	1	2	1	20
0	0	0	1	1	1	2	2	2	1	21
0	1	0	1	1	2	2	2	1	1	22

CD4 clones

CD3 clones

4	7	19	20	21	1	9	12	16	17	
1	0	1	1	1	0	0	0	0	0	2
2	2	1	1	1	0	0	1	0	0	6
2	2	1	0	1	0	0	0	0	0	16
0	2	2	2	1	0	0	0	0	0	17
1	2	1	2	2	0	0	0	0	0	18
1	1	0	2	1	0	0	0	0	0	19
0	1	2	2	1	0	1	0	0	0	20
1	2	1	2	1	0	0	0	0	0	21
2	1	0	1	1	0	0	0	0	0	22

CD4 clones

Thirdly, when young, non-cloned gametophytes are isolated, self-fertilisation regularly occurs and the variable lack of complete fertility may be ascribed to the incidence of sporophytic lethals for which the parent plant is heterozygous (Table 4). Thus, cloned and non-cloned gametophytes appear to differ dramatically in their capacity for self fertilisation. It was this contrast that prompted Klekowski (1972) to invoke the hypothesis of balanced lethals and suggest the possibility of atypical behavior in the populations studied by Wilkie (1956). Since the behavior of the cloned gametophytes is essentially the same in samples belonging to different taxa, or derived from geographically

TABLE 10. Frequency of formation of at least one sporophyte in random combinations of pairs of cloned gametophytes derived from the same or different spore samples.

Samples	N	Sporophyte production +	−	Frequency
i. *fulvum*—Clunie Dam				
CD2	50	24	26	0.48
CD3	50	24	26	0.48
CD4	50	30	20	0.60
Total	150	78	72	0.52
ii. *fulvum*—Clunie Dam				
CD2 and CD3	50	38	12	0.76
CD2 and CD4	50	22	28	0.44
CD3 and CD4	49	28	21	0.56
Total	149	88	61	0.59
iii. *aquilinum* and *fulvum*—Clunie Dam				
CD5 and CD2	25	25	0	1.00
CD5 and CD4	25	24	1	0.96
CD1 and CD5	25	21	4	0.84
Total	75	69	5	0.92
iv. *aquilinum* and *revolutum*				
BH3 and SL4	50	48	2	0.96

remote sites, it is likely that it holds for the *Pteridium* complex generally under the conditions provided in these experiments. At first sight the appearance of multi-allelic incompatibility looks like the obvious interpretation of the results of the diallel tests and this interpretation certainly cannot be excluded, although it encounters the embarrassing evidence for self fertilisation on the part of isolated, single non-cloned gametophytes. If gametic incompatibility does account for the reproductive behavior of cloned gametophytes it appears necessary to infer that the process of cloning has altered physiology or development to uncover an incompatibility system which is not normally expressed in non-cloned gametophytes.

However, it is necessary to enquire whether the apparent contradiction in the behavior of cloned and non-cloned gametophytes might be resolved within the framework of what is known about the reproductive behavior of gametophytes. Näf (1958) concluded that gametophytes form antheridia in response to antheridogen secreted into the medium by other, more rapidly growing gametophytes. If this external stimulus is absent, antheridia do not form although archegonia do. Hence, if a gametophyte develops from a single spore in isolation, it will be unable to undergo self-fertilisation. This appears to hold generally although exceptions may occur, especially after long periods of isolation.

It may be assumed that a cloned gametophyte behaves the same way and similarly requires an external stimulus to produce antheridia. The minimum requirement is the presence of another gametophyte that can provide the

TABLE 11. Diallel tests on cloned gametophytes of Clunie Dam samples (CD1 and CD5) of *aquilinum*. One combination (1 × 7) was lost due to algal infection.

CD1 clones										
1	3	7	11	14	12	2	5	8	9	
0	0	-	0	0	2	2	3	2	4	1
	0	0	2	0	0	4	2	3	2	3
		0	0	0	2	1	4	1	4	7
			0	0	2	4	4	1	1	11
				0	0	3	2	2	2	14
					0	3	2	3	1	12
						0	0	0	0	2
							0	0	0	5
								0	1	8
									0	9
CD5 clones										
2	5	13	17	10	11	12	20	4	16	
0	0	0	1	1	2	3	3	1	1	2
	0	0	0	1	3	3	3	3	3	5
		0	0	2	3	4	4	3	4	13
			2	1	3	4	3	3	3	17
				0	2	3	3	4	3	10
					0	1	2	0	0	11
						1	1	0	2	12
							1	0	1	20
								0	2	4
									0	16

stimulus, but not any gametophyte can provide it. The diallel tests suggest that the stimulus is generally present only when the two gametophytes concerned are genetically different. Since antheridogen is accepted as the agent which induces antheridia formation, one might wonder whether, within a species, it constitutes a single chemical entity or might occur in different forms which can be recognized by a gametophyte as different from the form it secretes, in which case only exposure to a different form would allow antheridia formation and hence the possibility of sporophyte formation. An alternative model could be envisaged in which the antheridogen is constant but other compounds take over the role just suggested, except that they would be responsible for determining whether or not a gametophyte responded positively to the presence of antheridogen.

At least, this hypothesis may have the merit of resolving the discrepancy between the behavior of sister clones and isolated non-cloned gametophytes and removes the need to invoke balanced lethals. Although separated at an early age, it is likely that the latter have already been primed to produce antheridia via exposure to the chemical stimuli contributed by genetically different gametophytes on the agar plate.

A number of Wilkie's (1956) results can be accommodated within this general scheme. Thus single, isolated gametophytes, derived from single spores by micro-manipulation, only rarely gave rise to a sporophyte. When